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METHODS AND APPARATUS USED IN IDENTIFYING LARGE NUMBERS OF LEAFHOPPERS OF THE GENUS EMPOASCA

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During the course of investigations on the potato leafhopper, *Emposaca fabae* (Harris), and closely related species, it has been necessary to identify accurately large numbers of specimens belonging to the genus *Empoasca*. Most of the species of this genus cannot be definitely differentiated by external morphological characters, and the chitinous structures of the abdomen, especially those in the male genital chamber, must be relied upon for this purpose. These characters are concealed and cannot be revealed and examined without special preparation of the chitinous structures, involving the destruction of the softer parts of the male abdomen by soaking gradually in a 10-percent solution of potassium hydroxide. Over 25,000 specimens of *Empoasca* have been specifically identified by this method at Arlington Experiment Farm during the period 1930-1935 in connection with studies of life history, feeding habits, host plants, host preferences, seasonal occurrence, and relative abundance, and of collections made in light traps, wind traps, and other experiments.

In order to facilitate the identification of large numbers of *Empoasca* it became necessary to develop special equipment and to adopt a uniform method of procedure in order to keep both the insect material and the records on it arranged so that they could be referred to promptly and conveniently at any time. The procedure developed is as follows: The first step is to detach the abdomen and place it in a small porcelain crucible (6 ml. capacity) containing a 10-percent solution of potassium hydroxide. These crucibles are placed in a pasteboard box, 9 by 12 inches in size (fig. 1), the bottom of which is fitted with a board containing holes into which the crucibles are set in order to hold them and prevent them from overturning. Each box holds 32 crucibles, and four boxes are used, thus providing for the soaking of the abdomens of 128 individual males at one time. The importance of individual treatment of each specimen should be emphasized because specific identification by external characters cannot be relied upon. The main purpose of the method is to keep each adult isolated and to arrange in systematic order the respective parts of each individual, during the process of identification, so that these parts can be readily located as desired and associated with the individual to which they belong.

The crucibles have porcelain covers which aid in preventing evaporation. The boxes are covered with pasteboard lids to facilitate handling and to exclude dust. An identification number is placed on the board in the box at the right of each crucible (fig. 1), for use while the abdomen is being soaked in the potassium hydroxide. The remainder of the adult to which the abdomen belongs is placed in a 2-dram homeopathic vial (fig. 2), the cork of which is numbered to correspond with the number at the right of the porcelain crucible containing the abdomen. After each specimen has been thus treated, notes on cards (3 by 5 inches), which are later filed in chronological order, are made opposite each corresponding number, as illustrated in figure 3, giving the information pertinent to the specimen. When the abdomen has been sufficiently cleared in the potassium hydroxide it is removed to a glass cell (such as is used in hydrogen ion testing sets) which contains a few drops of glycerine. The high refractive index of these glass cells containing glycerine makes them excellent for use in studying the insect by reflected light under the microscope. These glass cells are placed near the numbers in the box, opposite each crucible from which the abdomen was taken (fig. 1). After the specimen has been permitted to adjust itself (float out) in the glycerine, it is magnified from 60 to 115 times when it is studied under the binocular microscope. The name of the species, when determined, is then recorded on the cards as indicated in figure 3.

When it is desired to retain the specimen temporarily for further study, a small gummed label (bearing the date of identification and the serial number) is pasted on the side of the little glass cell containing the abdomen. These cells are kept in 2-ounce salve boxes for which a special holder is constructed (fig. 4) in order to provide for a systematic arrangement of this material and also to reduce the hazard of jarring and overturning these boxes and their contents. Paper-covered tops on the 2-ounce salve boxes are convenient for labeling and recording other identification data.

At the time the glass cell containing the cleared abdomen of a particular specimen is retained for further study, the remainder of this specimen in a homeopathic vial which bears the same number is set aside and labeled with the identification date and serial number.

When a permanent mount is desired, the adult is mounted on a cardpoint and labeled in the usual way to be placed in a Schmitt box for reference (fig. 5). The cleared abdomen of each adult is then placed in a small vial (10 mm. by 4.5 mm., outside dimensions) containing a few drops of glycerine (about one-third full). This is attached to the same pin, directly beneath the cardpoint, by pinning through the cork as illustrated in the insert of figure 5. On the bottom of the collector label are written the date of identification and the serial number, so that specimens mounted on pins can be arranged according to species by referring to the notes without re-examining the cleared abdomens, even though the species labels may become confused. A specimen cannot be identified successfully by examining the cleared abdomen through the curved glass of the small vial.

Figure 5 also illustrates a collection of permanent mounts of Empoasca in a Schmitt box. The pill box shown in the lower right hand corner of the box contains paradichlorobenzene which is used as a fumigant. A thumb tack is used to attach the bottom of the pill box to the inside of the Schmitt box. The lid of the pill box, which is perforated with numerous small holes through which the gas may escape, is securely fitted or glued in place.

The method of making permanent mounts described here has been in use more than three years without the necessity of refilling the vials with glycerine. Permanent slide mounts of the cleared abdomens are unsatisfactory for specific identification of many of the species of Empoasca. It is frequently necessary to study the male genitalia from various angles because only the relative position of the genital pieces floating in the liquid makes possible specific identification.

EXPLANATION OF FIGURES

Figure 1.-- Pasteboard box with wooden board for holding crucibles which contain a 10 percent solution of potassium hydroxide; the lids for the crucibles in the first row, left, are removed to the left side of the box. Small glass cells at the right of the crucibles contain glycerine, into each of which the cleared abdomen of an individual Empoasca is placed for study under the microscope.

Figure 2.-- Box of 2-dram homeopathic vials, each containing an Empoasca adult from which the abdomen has been removed for clearing in potassium hydroxide. Each adult is identified by the number on the cork of the vial into which it is placed, and its abdomen is placed in a crucible bearing the corresponding number (as shown in fig. 1).

Figure 3.-- Illustration of method used to keep records on material studied for identification.

Figure 4.-- Holder for sixteen 2-ounce salve boxes in which are stored small glass cells containing cleared abdomens of Empoasca in glycerine. This method provides a systematic arrangement of material and insures safer handling.

Figure 5.-- Permanent mounts of Empoasca in Schmitt box. Insert: A permanent mount enlarged to show detail. This mount was arranged to suit the focal plane of the lens of the camera rather than to illustrate the exact relative position of the labels, vial, and cardpoint on the pin. The pin is inserted through the cork so that the cleared abdomen in the small amount of glycerine remains in the bottom of the vial while permanently stored

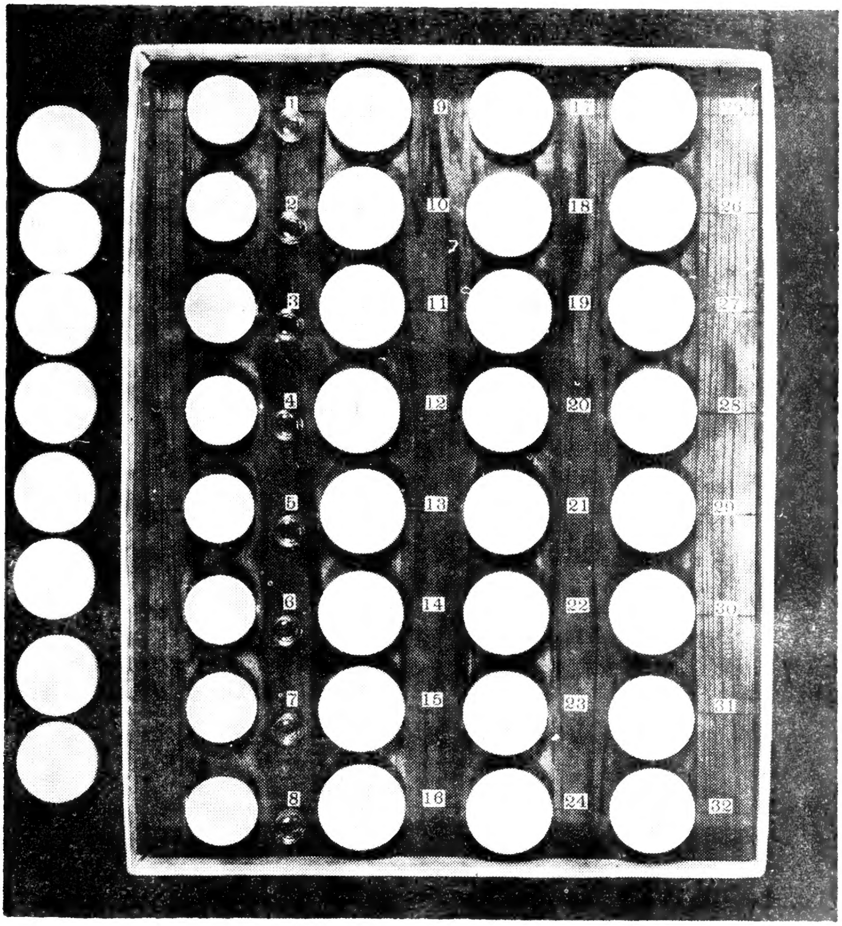


Figure 1.--Pasteboard box with wooden board for holding crucibles.

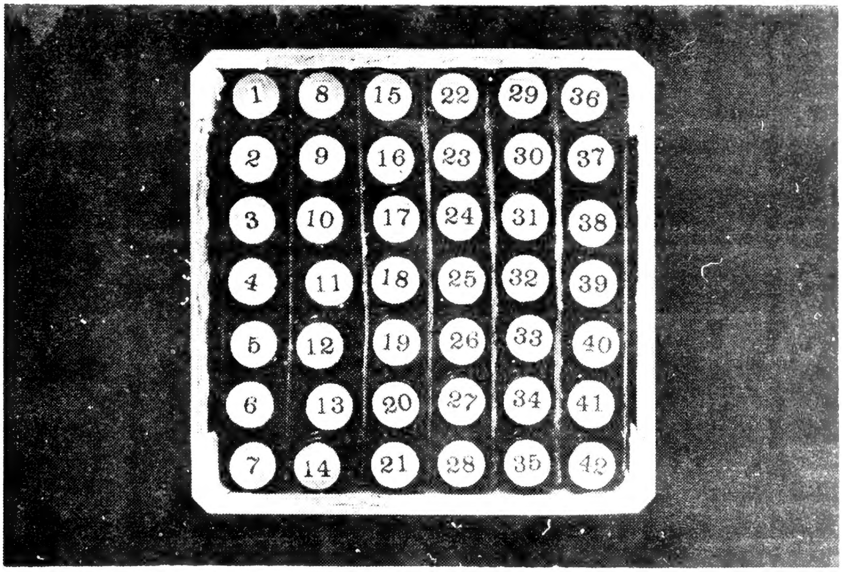
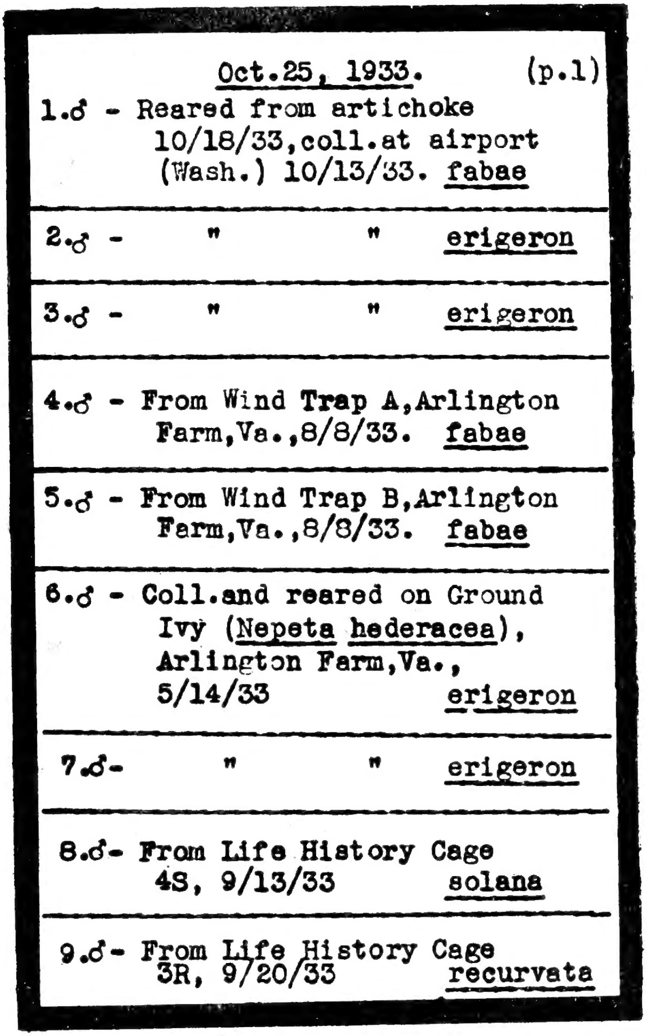


Figure 2.--Box of 2-dram homeopathic vials.



Oct.25, 1933. (p.1)

1.♂ - Reared from artichoke 10/18/33, coll. at airport (Wash.) 10/13/33. fabae

2.♂ - " " erigeron

3.♂ - " " erigeron

4.♂ - From Wind Trap A, Arlington Farm, Va., 8/8/33. fabae

5.♂ - From Wind Trap B, Arlington Farm, Va., 8/8/33. fabae

6.♂ - Coll. and reared on Ground Ivy (Nepeta hederacea), Arlington Farm, Va., 5/14/33 erigeron

7.♂ - " " erigeron

8.♂ - From Life History Cage 4S, 9/13/33 solana

9.♂ - From Life History Cage 3R, 9/20/33 recurvata

Figure 3.--Illustration of method used to keep records.

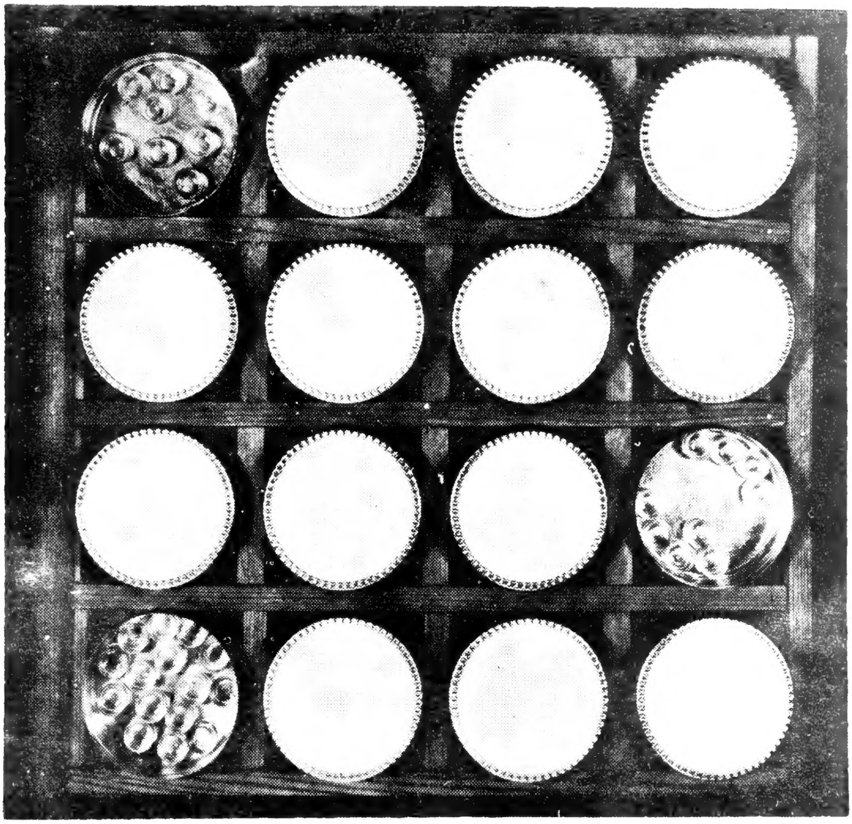


Figure 4.--Holder for sixteen 2-ounce salve boxes.

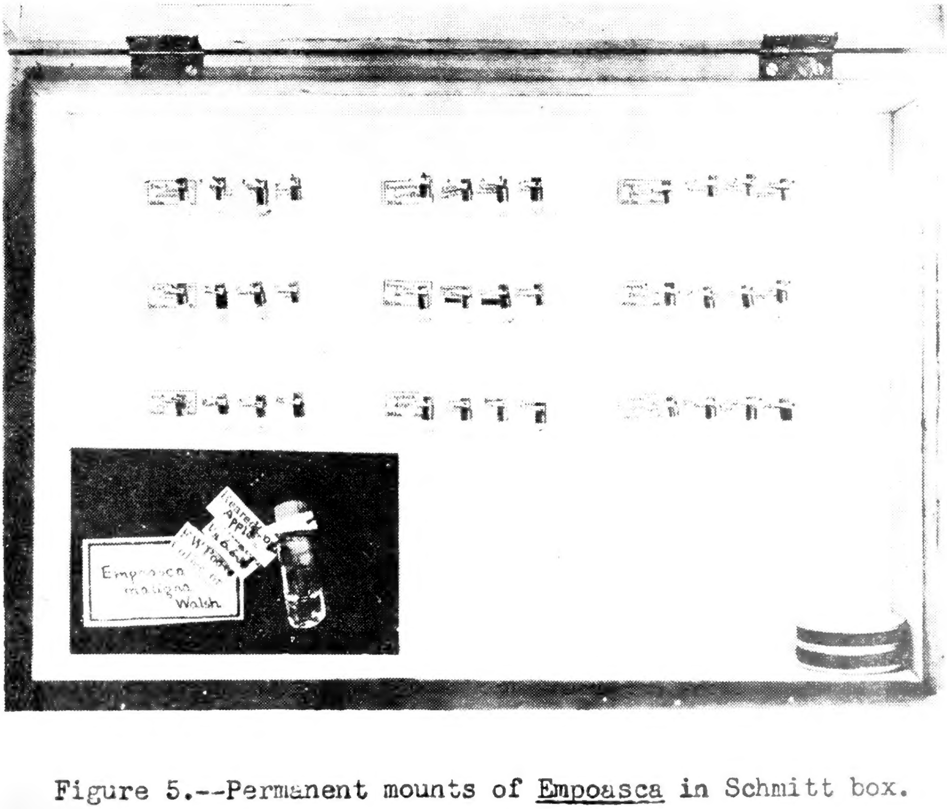


Figure 5.--Permanent mounts of Empoasca in Schmitt box.